Peer reviewed article

Seroimmunity to diphtheria and tetanus among mother-infant pairs; the role of maternal immunity in infant immune response to diphtheria-tetanus vaccination

Mohammed-Jafar Saffar[a], *Ali-Rezas Khalilian*, *Abolghasem Ajami*, *Hiva Saffar*, *Abbas Qaheri*

[a] Paediatric Infectious Diseases Ward, Boali-Cina Hospital, Mazandaran University of Medical Sciences, Pasdaran Boulevard, Sari-Iran

Summary

Background: This study was designed to determine the levels of immunity against diphtheria and tetanus in 110 mothers with/without diphtheria-tetanus toxoid (dT) vaccination during pregnancy and their two-month-old infants before diphtheria-tetanus-pertussis (DTP) immunisation, and also to assess the influence of pre-vaccination passive immunity on the infants' immune response to three doses of DTP vaccination.

Subjects and methods: Sera from 110 mother-infant pairs before DTP vaccination and from 69 infants after receipt of three doses of DTP vaccine were tested to measure antidiphtheria-antitetanus toxin IgG levels, using a commercial enzyme immunoassay. History of dT toxoid vaccination of mothers at pregnancy was recorded.

Results: 20% of mothers did not receive dT vaccine. Among these 22 unvaccinated mothers, one (5%) and six (27%) were serologically susceptible to tetanus and diphtheria respectively. The mean concentrations of antibody titers were lower in unvaccinated than in vaccinated mothers: diphtheria 0.78 (0.30) IU/mL *vs* 0.31 (0.20), and tetanus 1.95 (1.20) IU/mL *vs* 0.51 (0.45), vaccinated mother *vs* unvaccinated. All infants (100%) acquired immunity against both infections after receipt of three doses of DTP vaccine. Pre-vaccination passive immunity did not influence the infants' immune response to vaccination: diphtheria 0.95 (0.40) *vs* 0.89 (0.25), and tetanus 2.30 (1.0) *vs* 2.30 (0.70), from passive immune infants before vaccination *vs* those without, respectively.

Conclusion: This study showed that diphtheria-tetanus toxoid components of DTP vaccine were highly immunogenic and maternal passive immunity did not affect the infants' immune response to DTP vaccination. Since there is a 23% missed opportunity for dT immunisation, efforts must be made to increase the coverage rate of this highly immunogenic vaccine in order to sustain protection against diphtheria and tetanus in mothers and their infants.

Key words: diphtheria; DTP immunogenicity; mother-infant pairs; pregnancy; tetanus

Introduction

Maternal immunisation offers a potential means of protecting infants against infection by a number of important pathogens during the first few months of life, if universally applied [1]. Many countries routinely immunise mothers during pregnancy with tetanus toxoid containing vaccine [2, 3]. Maternally transferred antibodies play an important role in infant protection. However, some studies suggest that maternally originated antibodies in infants at the time of immunisation are linked to failure of infants' immune response to the vaccine [4].

The Iranian Advisory Committee on Immunisation Practices (ACIP) has implemented the WHO expanded programme on immunisation (EPI) recommendations [5] as a primary course of combined diphtheria-tetanus-pertussis (DTP) vaccine in childhood (at age 1.5, 3 and 4.5 months, amended recently to 2, 4 and 6 months, with two additional doses at the age of 18–24 months and 4–6 years), plus booster doses containing only diphtheria and tetanus toxoids (dT) every 10 years thereafter. Also, to prevent puerperal and neonatal tetanus, national guidelines call for screening and vaccination of eligible women during pregnancy. Pregnant women

This project was funded by the Deputy Ministry for Food and Drugs, Ministry of Health and Medical Education IR Iran, and the Vice-Chancellor for Research of Mazandaran University of Medical Sciences.

whose vaccination status is unknown or who received less than three doses of DTP/dT vaccine during their lives should receive two doses of dT vaccine at the 4th and 6–7th month of pregnancy. This schedule has been implemented since 1983 in Iran and the vaccine coverage rates exceeded 95% some years later. This programme has been a tremendous success and cases of diphtheria and tetanus are now at their lowest levels, with only 159 cases of diphtheria and 81 cases of tetanus (one neonatal tetanus) reported in Iran from 1995–2000.

This study was conducted to determine the dT vaccine coverage rate, the seroprevalence and titres of antidiphtheria and antitetanus antibodies among mothers and their two-month-old infants before administration of the first dose of DTP vaccine, and its relation to dT vaccination status of mothers during pregnancy. Also, assessment of the infant's immune response to the DT component of DTP vaccine after administration of three doses, and the influence of maternal antibodies on infants' immune response to the vaccine, were among the other important objectives of this study performed in Sari-Iran.

Subjects and methods

This study was conducted from February 2005 to July 2006 at the primary health centres affiliated to Sari Medical Faculty. Study subjects were apparently healthy mothers and their two-month-old infants who were brought for scheduled routine primary immunisation with DTP vaccine at the ages of 2,4, and 6 months (DTP vaccine: diphtheria toxoid 15 Lf, tetanus toxoid 10 Lf. Whole-cell pertussis 16 protective units; Razi Institute, Iran). The medical health charts of all mothers presenting to the health centres for routine DTP immunisation of their infants were reviewed to recruit those who followed the national guidelines for dT vaccination in pregnancy. All cases were apparently healthy with a normal physical examination at the time of immunisation, and were consecutively selected. Infants with histories of prematurity or birth weight <2500 gr, neurological problems or undefined seizure disorders, acute febrile or non-febrile illnesses, recipients of blood, blood products or immunoglobulin, and with evidence of immunodeficiency in either mothers or infants were excluded. Mothers with a previous history of complete DTP/DT/dT vaccination were also excluded. The Mazandaran University Research Ethics Committee and the Iranian Ministry of Health Review Board approved this project. The study objectives were explained to the parents before obtaining written consent from the study participants and infants' parents/guardians. Blood samples from each mother-infant pair were taken just before the first dose and only from infants 4–8 weeks after administration of the third dose of DTP vaccine. Sera were stored at –20 °C until assayed at the same time at the University laboratory. Antidiphtheria and antitetanus antitoxin titres were measured quantitatively using an enzyme immunoassay (ELISA) method employing diphtheria IgG and tetanus IgG ELISA kits (IBL immunobiological laboratories, IBL, Hamburg-Germany) based on the sandwich principle and manufacturer's instructions. The cut-off values of <0.01 IU/mL and <0.05 IU/mL were considered negative (susceptible), 0.011 to 0.50 and 0.050 to 0.5 as protected, and above 0.5 and >0.5 IU/mL as highly protective titres for diphtheria and tetanus respectively. Immunological response to the vaccine was defined as changing seronegative sera before vaccination to positive and/or a 4-fold increase in antitoxin titres after vaccination. Based on dT (dT-adult, diphtheria toxoid 2Lf, tetanus toxoid 10 Lf, Razi Institute, Iran) and vaccination status of mothers, they and their infants were divided into two groups (dT positive Vs dT negative). Mean concentration of antitoxin (MCA) titres and the immune status of the subjects based on antitoxins levels were calculated for mothers and infants before the first dose and in infants after the third dose of the DTP vaccine in both groups, and were compared with each other.

Results

During the study period, a total of 585 mother-infant pairs were presented to health centres for scheduled infant immunisation. Of this total 46 infants (8%) did not fulfill the criteria and were excluded. 113 mothers were likewise excluded due to a previous history of complete primary series of DTP/dT immunisation. Of 426 mothers eligible for dT vaccination, 98 (23%) did not receive dT vaccine. However, of these candidate mother-infant pairs 110 (26%) were accepted for inclusion in this study. The mean age of the mothers was 26 (5) years and of the infants 65 (7) days. Although the mothers' history of dT vaccination during their last pregnancy was clear, their previous history of primary series of DTP immunisation during childhood was not well documented. However, they could recall some vaccine injections during childhood with help from their mothers (infants' grandmothers).

Review of the mothers' medical charts showed that 23% of candidate mothers did not receive dT vaccination, the main cause being a tendency of mothers to seek care from obstetrician-gynaecologists in private clinics instead of primary health centres. As shown in table 1, of 110 mothers studied, 88 (80%) had received dT vaccine during their last pregnancy. There were differences between dT vaccinated and unvaccinated mothers regarding antidiphtheria and antitetanus antitoxin MCA levels as well as the three cate-

gories immunity strength, antidiphtheria seroprevalence rate: 100% *vs* 73%, and MCA titers of 0.78 (0.30) *vs* 0.31 (0.20) IU/mL, antitetanus seroprevalence rate 100% *vs* 95%, and MCA levels 1.95 (1.20) *vs* 0.51 (0.45) IU/mL. These rates for infants were as follows: antidiphtheria seroprevalence rate 89% *vs* 50%, MCA 0.50 (0.30) *vs* 0.17 (0.15) IU/mL, and antitetanus seroprevalence rates 81% *vs* 68%, MCA titres 1.16 (1.20) *vs* 0.31 (0.25) IU/mL, from dT vaccinated *vs* non-vaccinated mothers respectively (table 2).

For the second stage, 69 infants (52 from dT vaccinated mothers) with a mean age of 220 (25) days were brought in to complete the study. The major reason for dropout was parental refusal to allow further blood sampling. After administering three doses of DTP vaccine, all infants responded to vaccination and obtained full protection against diphtheria and tetanus (4% protection and 96% full protection). As shown in table 3, there were no apparent differences between the two groups of infants (infants with passive immunity before vaccination *vs* those without it) in response to vaccination, diphtheria 0.95 (0.40) *vs* 0.89 (0.25) IU/mL, and tetanus 2.30 (1.0) *vs* 2.30 (0.70), for infants with and without passive immunity respectively.

Table 1

Seroimmunity rates and mean concentration of antibody titers against diphtheria and tetanus in mothers and their relation to mothers dT[1] vaccination status, Sari-Iran.

	dT[1] positive mothers no = 88	dT[1] negative mothers no = 22
Diphtheria		
MCA titres[2] (SD) IU/mL	0.78 (0.30)	0.31 (0.20)
Susceptible: <0.050 IU/mL	0	6 (27%)
Protected: 0.051–0.50 IU/mL	19 (22%)	12 (55%)
Fully protected: <0.50 IU/mL	69 (78%)	4 (18%)
Tetanus		
MCA titres[2] (SD) IU/mL	1.95 (1.20)	0.51 (0.45)
Susceptible: <0.010 IU/mL	0	1 (5%)
Protected: 0.011–0.50 IU/mL	11 (13%)	13 (59%)
Full protected: >0.50 IU/mL	77 (87%)	8 (36%)

[1] Adult type diphtheria-tetanus vaccine, [2] Mean concentration of antibodies

Table 2

Seroimmunity levels and mean concentration of antibody titres against diphtheria and tetanus in infants and their relation to their mothers' dT[1] vaccination status, Sari-Iran.

	dT[1] positive infants no = 88	dT[1] negative infants no = 22
Diphtheria		
MCA titers[2] (SD) IU/mL	0.50 (0.30)	0.17 (0.15)
Susceptible: <0.050 IU/mL	10 (11%)	11 (50%)
Protected: 0.051–0.50 IU/mL	25 (29%)	11 (50%)
Fully protected: <0.50 IU/mL	53 (60%)	0
Tetanus		
MCA titres[2] (SD) IU/mL	1.16 (1.20)	0.31 (0.25)
Susceptible: <0.010 IU/mL	17 (19%)	7 (32%)
Protected: 0.011–0.50 IU/mL	19 (21%)	13 (59%)
Fully protected: >0.50 IU/mL	52 (60%)	2 (9%)

[1] Adult type diphtheria-tetanus vaccine, [2] Mean concentration of antibodies

Table 3

Immunogenicity[1] of diphtheria and tetanus components of combined diphtheria-tetanus and pertussis vaccine (DTP) after administration of three doses of DTP vaccine and its relation to maternal passive immunity in 69 infants.

	dT[2] positive infants no = 52	dT[2] negative infants no = 17
Diphtheria MCA[3] levels (SD) IU/mL		
Before first dose	0.37 (0.30)	0.18 (0.15)
After three doses	0.95 (0.40)	0.89 (0.25)
Tetanus MCA[3] levels (SD) IU/mL		
Before first dose	0.97 (1.10)	0.25 (0.25)
After three doses	2.30 (1.0)	2.30 (0.70)

[1] All infants from both groups responded and acquired immunity,
[2] Adult type diphtheria-tetanus vaccine, [3] Mean concentration of antibodies

Discussion

Considering the Iranian ACIP guidelines, this study showed that 23% of eligible mothers were not vaccinated during pregnancy, and the MCA levels of their antitoxin antibodies against diphtheria and tetanus were lower than in vaccinated mothers. The main cause of this missed opportunity was women's frequent failure to visit primary health centres for care during pregnancy, presenting to private clinics instead.

In a similar study to determine the proportion of pregnant women who followed the national guidelines for vaccination, Goncalves et al. [6] studied susceptibility to tetanus and missed vaccination opportunity in 96 Portuguese pregnant women by the ELISA method. They showed that 50% of eligible women were not vaccinated, 15% of these had IgG antitoxin antibody levels below titres considered protective, and antibody levels were significantly lower in non-vaccinated women than in those vaccinated during pregnancy.

Mass vaccination during childhood and in pregnancy has been very successful in most countries, where the incidence of most vaccine-preventable infections has decreased markedly [7]. Because of this, most parents, families, and health personnel (especially of the younger generation) may be unfamiliar with these infections and their complications. Where the severity of these diseases is not appreciated, apathy may decrease the vaccine coverage rates in the target population and detract from the success of control programmes [8].

As concluded by Goncalves et al. [6] and also based on the results of our study, because of high rates of missed immunisation opportunities, efforts to sustain high vaccine coverage and encourage observance of national guidelines for pregnant women must be intensified. Health personnel and obstetrician-gynaecologists (who may be the sole primary care providers for pregnant women) must be made aware of the guidelines and the importance of implementing them when necessary. Women must be educated and motivated to demand vaccination [9].

In this study susceptibility to diphtheria in unvaccinated mothers was 27%, but in contrast to low rates of immunity to diphtheria, all unvaccinated mothers except one were serologically immune to tetanus infection. The true causes of this high rate of seroimmunity to tetanus are not clear but can be explained as follows: (a) tetanus toxoid vaccination following injuries, (b) dT boosting at highschools in Iran (since 1993 passive dT immunisation with low coverage rate (<30–50%) has operated in highschools), and (c) persistence of acquired immunity following primary series of DTP vaccination in childhood; these cannot be verified, however. The rates of immunity to diphtheria and tetanus in unvaccinated mothers in our study were similar to the findings of a study on Romanian mothers [10]. In a study on antidiphtheria and antitetanus immunity in Romanian mother-infant pairs with and without tetanus toxin (TT) vaccination in the 7th month of pregnancy, Durbaca showed high rates of antitetanus immunity in mother-infant pairs (94% and 93% respectively). However, similarly to our study, immunity to diphtheria was low (80% and 77%). The antitetanus immunity rate was 100% in TT vaccinated mothers and their infants.

The results of many serological studies on immunity to diphtheria and tetanus carried out worldwide indicate that vaccine-induced immunity by childhood immunisation decreases with advancing age in the absence of boosting [11]. This may account for the lack of protective antibody levels in some vaccinees. In a study by Kruszon-Moran et al. [9] on diphtheria and tetanus immunity among U.S females, it was shown that more than half of 20-year-old women were not protected fully against these infections. In another study on immunity to these infections in England and Wales [12], the results indicated that >20% of 2 to 20–24-year-old and >20% of 4 to 30–34-year-old individuals were not protected against diphtheria and tetanus respectively. Results also reveal that levels of immunity decreased with advancing age. The results of many other studies on seroimmunity to diphtheria [13] and tetanus [14] are in accord with our findings.

Maternal antibody-mediated inhibition of immune response to vaccine has been described for live and non-live vaccines. However, contradictory reports have resulted in a confusing situation with regard to the relative biological and clinical significance of this phenomenon [4]. A study by Bjorkholm et al. [15] revealed an inhibitory effect of maternal antibody on immune response to diphtheria toxoid vaccine. The conclusion in a study by Saras et al. [16] was early suppression effects by maternal antidiphtheria and antitetanus antibodies, but the final immune response to a course of 2–3 doses of DTP vaccine was not affected by passive immunity. As shown in this study, infants' final immune response to three doses of DT components of DTP vaccine was satisfactory and not influenced by pre-vaccination antibody levels in infants, while all vaccinees in both groups responded to vaccination and acquired immunity against diphtheria and tetanus.

Conclusion

This study revealed that the diphtheria and tetanus toxoid components of DTP vaccine were highly immunogenic, and infants' passive immunity before vaccination did not affect their final immune responses to the vaccination. Also, this study showed that there was a 23% missed opportunity for dT vaccination, and that efforts must be made to increase the coverage rates of this highly immunogenic vaccine in order to sustain protection against diphtheria and tetanus in mothers and their infants.

We thank the mothers and their infants for their participation, the health care workers for their co-operation and Dr Abdollahi A for data collection. Special thanks are due to Dr Enayati for his critical reading of the manuscript.

Correspondence:
Mohammed-Jafar Saffar
Pediatric Infectious Diseases Ward
Boali–Cina Hospital
Mazandaran University of Medical Sciences
Pasdaran Boulevard
Sari-Iran
E-Mail: saffar@softhome.net

References

1 Healy CM, Beker CJ. Prospect for prevention of childhood infections by maternal immunisation (pediatric and neonatal infections). Curr Opion Infect Dis. 2006;19(3):271–6.
2 World Health Organization, Expanded programme on immunisation. Immunisation policy. Geneva; 1996 [WHO/GEN/95.03 Rev.1].
3 Black RE, Huber DH, Curlin GT. Reduction of neonatal tetanus by mass immunisation of non-pregnant women: duration of protection provided by one or two doses of aluminum absorbed tetanus toxoid. Bull World Health Organ. 1980;58: 927–30.
4 Siegrist C-A, Cordova M, Brandt C, Barria C, Berney M, Tougne C, et al. Determinant of infant responses to vaccines in the presence of maternal antibodies. Vaccine. 1998;16(14/15): 1409–14.
5 Expanded programme on immunisation, Global Advisory Group. Wkly Epidemiol Rec. 1981;56(2):9–16.
6 Goncalves G, Santos MA, Cutts FT, Barros H. Susceptibility to tetanus and missed vaccination opportunities in Portugueze women. Vaccine. 1999;17:1820–5.
7 Dennehy PH, Peter G. Active immunizing agents. In: Feigin, Chery. Demmler, Kaplan textbook of pediatric infectious diseases, fifth ed. Saunders Philadelphia, 2004,P:3136–82.
8 Katz SL. Dublin reminds US about measles. Pediatric Infect Dis J. 2003;22(7):579.
9 Kruszon-Moran DM, McQuillan GM,Chu SY. Tetanus and diphtheria: immunity among females in the U.S: are recommendations being followed? Am J Obstet Gynecol. 2004;190: 1070–6.
10 Durbaca S. Anti-tetanus and anti-diphtheria immunity in newborns. Roum Arch Microbiol Immunol. 1999;58(3,4):267–72.
11 Simonson O. Vaccination against tetanus and diphtheria: evaluation of immunity in the Danish population, guideline for revaccination, and methods for control of vaccination programs. Danish Med Bull. 1989;36:24–47.
12 Maple PAC, Jonson CS, Wall EC, Vyse A, Edmunds WJ, Andrews NJ, et al. Immunity to diphtheria and tetanus in England and Wales. Vaccine. 2001;19:167–73.
13 Chironna M, Germinario C, Lopalco PL, Carrozinni F, Barbuti S, Quarto M. Immunity to diphtheria: immunity among refugees in southern Italy. Vaccine. 2003;21:3157–61.
14 Gergen PJ, McQuillan GM, Kiely M, Ezzati-Rice TM, Sutler RW, Virella G. A population based serological survey of immunity to tetanus in the U.S. N Engl J Med. 1995;332(12):761–6.
15 Bjorkholm B, Granstrom M, Taranger J, Wahl M, Hagberg L. Influence of high titers of maternal antibody on the serologic response of infants to diphtheria vaccination at three, five and twelve months of age. Pediatr Infect J Dis. 1995;14:846–50.
16 Sarvas H, Kurikka S, Seppala IJT, Makela PH, Makela O. Maternal antibodies partly inhibit an active antibody response to routine tetanus toxoid immunisation in infants. J Infect Dis. 1992;165(May):997–9.

Established in 1871

Formerly: Schweizerische Medizinische Wochenschrift

SMW Swiss Medical Weekly

The European Journal of Medical Sciences

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising. The 2006 impact factor is 1.346.
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of professional statisticians for every article with statistical analyses

- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts:	submission@smw.ch
Letters to the editor:	letters@smw.ch
Editorial Board:	red@smw.ch
Internet:	http://www.smw.ch

EMH FMH Schwabe
Editores Medicorum Helveticorum

Official journal of the Swiss Society of Infectious Diseases, the Swiss Society of Internal Medicine and the Swiss Respiratory Society

Supported by the FMH (Swiss Medical Association) and by Schwabe AG, the long-established scientific publishing house founded in 1488